REMARKS/ARGUMENTS

The Final Office Action dated 6/19/2007 rejected claims 1-8 and 18; claims 9-17, 20 and 21 were withdrawn from consideration. No claims were allowed. By this Amendment, claims 1, 4, and 18 are amended and claim 5 is canceled.

This Amendment and Response is being submitted within two months of the mailing date of the Final Rejection. Calculation of a shortened statutory period for reply under MPEP 714.13 is requested. In the event that the Office does not mail an advisory action until after the end of the three month period set in the final office action extension of time fees will be based on the date of mailing of the advisory action.

Entry of the present amendment is respectfully requested under 37 CFR 1.116 and MPEP 714.13, which provides: "The proposed amendment should be given sufficient consideration to determine whether the claims are in condition for allowance and/or whether the issues on appeal are simplified." The present amendment is intended not to touch on the merits of the final rejection, but to limit the claims to those indicated as allowable in the previous office action.

Turning now to the Detailed Action, the following remarks are set forth and responded to in the same order as presented in the Office Action.

Claim Rejections - 35 USC §112 (Office Action Paragraphs 2-3)

The specification was said to be "enabling for truncated extracellular portion of mouse Type1 IP3R comprising at least amino acids sequence of 226-578," but it does "not reasonably provide enablement for all any truncated portion (sequence) of IP_3R as binding protein which would have an affinity of at least about 200 times the affinity for 1P3 than that of intact 1P3R for IP_3."

Response

Claims 1 and 18 and claims dependent thereon have been amended to restrict the truncated protein to the protein used in the working exemplification to further the prosecution of this application and put the application in form for allowance. Support for

the new language is found in paragraphs [00041] and [00051] (claim 1) and [00051] (claim 4). The rejection under 35 USC §112, 1st paragraph is submitted to have been avoided and the Examiner is respectfully requested to withdraw this rejection.

Claim Rejections 35 USC §103 (Office Action Paragraphs 4-6)

Claims 1-4, 6-8 and 18-19 were rejected over Sportsman et al. in view of Iwaskaki et al. and Hirata et al. Claim 5 was rejected over the preceding combination of references further in view of Henderson (Office Action paragraph 17).

Office Action paragraph 6, page 6, states:

"Since labeling analytes with enzyme fragment (enzyme donor conjugate i.e. tracer) is common and known for its sensitive detection and are not limited to small molecular analytes (Henderson et al.), it would have been obvious to one of ordinary skill in the art at the time the invention was made to use enzyme fragment (e.g. donor fragment of beta-galactosidase) to label IP3 in the method of Sportsman et al. for detection of analytes with a reasonable expectation of success because production of enzyme fragment label conjugate and complementation assays are taught in the method of Henderson et al."

The Response to Arguments states:

"Sportsman et al. disclose that specific binding partner generally comprises any compound capable of specifically and competitively binding an analyte and an associated tracer and also disclose that fragments, derivatives or analogs of a preferred specific binding partner may be used (column 11, lines 22-35). Therefore, Sportsman et al. discloses strong motivation to involve other binding partners or fragments thereof in the assay methods. Iwasaki et al. and Hirata et al. are combined with Sportsman et al. because Iwasaki et al. disclose N- terminal 226-578 amino acid sequence of mIP3R1 binds to IP3 with high affinity and thus would be obvious to try as a binding partner as taught by Sportsman et al. and IP3 tracer having label attached to 2 hydroxy position of IP3 as taught by Hirata et al would be obvious in the assay method of sportsman et al. because Hirata et al. disclose labels attached to 2 hydroxyl position do not substantially interfere with the affinity of IP3 for IP3 receptor. Therefore, motivation is there to combine the references."

Response

The rejection relies on Sportsman et al. in view of Iwasaki et al. and Hirata et al. As for Iwasaki, since applicants use the "sponge protein" of Iwasaki, its relevance is admitted. The issue is whether it was obvious to combine Iwasaki's teaching with Sportsman and Hirata.

Considering Sportsman first, the relevant part of the reference is found in Example 14. This example shows assays for components of the inositol-phospholipid signaling pathway, including associated G-proteins. Generally, the assays include luminescence polarization assays directed to intermediates of this pathway, such as 1,4,5 IP3. The assays include a tracer form of the intermediate and a specific binding partner of the intermediate and tracer. The tracer may include a luminophore attached by a suitable chemistry to the intermediate (such as a fluorescein succinyl-labeled IP3). The binding partner may include an antibody that specifically binds to the intermediate and tracer. Assays may be performed as shown in FIG. 5, with the intermediate taking the place of the cyclic nucleotide. Assays for associated G-proteins may be performed as shown in FIG. 6. These assays may be used to measure the presence, concentration, and/or activity of intermediates, enzymes, and/or receptors involved, in this pathway, or they may be directed to associated tissues and responses, as indicated in the following table:

Selected Hormone induced cellular Responses Medicated by G-Protein-linked Receptors Coupled to the Inositol-Phospholipid Signal Pathway

Target Tissue	Signaling Molecule	Major Response
Liver	Vasopressin	Glycogen breakdown
Pancreas	Acetylcholine	Amylase secretion
Smooth muscle	Acetylcholine	Contractions
Mast cells	Antigen	Histamine secretion
Blood platelets	Thrombin	Aggregation

A number of aspects of this teaching should be considered. The example was never performed, the Figures being cartoons of the events for the assay. Nor was the "tracer"

prepared, since there are no directions as to how to prepare the tracer. IP3 having three hydroxyl groups, there is no direction whether one should randomly conjugate the fluorescein to the available hydroxyl groups or by some legerdemain, not indicated, protect two of the hydroxyl groups, conjugate the fluorescein to the available hydroxyl group and then remove the protecting groups without affecting the linker to the IP3. If the Examiner is citing this reference as indicating an interest in being able to assay for IP3, then the reference is appropriate for that basis. If the reference is being cited as suggesting a useful method for performing an assay for IP3, such an application goes far beyond what the reference can support.

To recapitulate, combining Sportsman and Iwasaki, one has a protein that binds IP3 with high affinity and a suggestion that one should make a detectable derivative of IP3 to perform an assay. The protein suggested in Sportsman is an antibody, not a truncated derivative of mouse IP3R.

The next reference is Hirata. Hirata prepares derivatives of IP3 and performs an assay for binding to rat cerebellum homogenate obtained as a dispersed pellet using radioactive IP3 as the tracer. There is no characterization of the homogenate as to the inositol phosphate receptors, the degree of non-specific binding of IP3 and the analogs, and the specificity for IP3R as compared to other proteins that bind IP3. Therefore, it is greatly a matter of conjecture as to what is occurring in Hirata's assays. The procedure is a simple competition between hot IP3 and cold IP3 or IP3 analogs. The assay determines how much of the hot IP3 remains bound to the microsomes retained by a glass fiber filter.

Looking at Table 6, indicated as a displacement assay, but really a competition assay, the dark round circles are the competition between the hot and cold IP3. When the concentration of the two components is the same, only about 10% of the hot IP3 is captured by the column. This suggests that the microsomes have an excess capacity for the IP3, since if there was an inadequate capacity, then one would expect about 50% of the hot IP3 would be retained as compared to the amount retained in the absence of the cold IP3. The

scale would appear to be a log scale that smoothes out the scatter and provides for some linearity. One cannot evaluate differences in binding of the various IP3 entities to different binding sites from the log scale.

One would expect that the hot IP3 would compete equally with the cold IP3 for the binding sites of the IP3R and other sites to which IP3 binds. There are different binding affinities between the isoforms of IP3 and significant cross-reactivity with other receptors that bind different phosphorylated derivatives of inositol. See, Vanlingen, et al 2001 Biochem Pharmacol 61, 803-9, Yamamoto-Hino 1994 Receptor Channels 2, 9 – 22 and Staudman et al 1988 Biochem J 255, 677 – 83. While there would appear to be no difference between the cold IP3 and its analogs in competing off the hot IP3, we do not know what binding sites are being competed off by these entities. Nor do we know to what extent non-specific binding is occurring, where non-specific binding would be expected to be readily displaceable.

It is noted that the L-isomers have little or no effect. This would suggest that there is specific binding of the IP3 and its analogs, rather than looking at a mass effect. However, there is no certainty as to what the various entities are binding to, except that there seems to be a concentration effect. That at 10 x the amount of analog as compared to hot IP3 one observes about a 50% reduction in binding of the hot IP3 is not showing a strong competitor, regardless of what the two compounds are binding to.

Finally, there is no knowledge as to what other proteins may be present that could affect IP3 and the analogs. For example, esterases could hydrolyze the analogs leaving only IP3, which could be further hydrolyzed by phosphatases. The analogs could be hydrolyzed by phosphatases. There are no controls to determine the fate of the analogs.

What does the example teach? What is reasonably taught by the example is that a homogenate of rat cerebellum—known to have IP3R and many other receptors for other IP analogs—will bind to IP3 and derivatives at the 2-position of IP3 or the hydrolytic products

thereof. The large number of available sites in the homogenate, the relatively low affinity that IP3 has for the different isoforms of IP3R, and the possibility of modification of the IP3 and its analogs by enzymatic reactions, makes the data very difficult to analyze.

In addition, there is the fact that in the subject assay the protein is not an intact IP3R. Rather as the Examiner has properly noted and is presently claimed, a fragment of the IP3R is used with a reported 10^3 increase in affinity for IP3. Regardless of the basis for the increased affinity, there must be some change in the conformation of the protein that so greatly enhances the binding properties. Since the "sponge" protein and the IP3R receptor from which it is derived have such an extraordinary difference in binding affinity, one must assume that the pocket in which the IP3 binds is different in the two proteins. It follows that one cannot predict the binding affinity of a derivative of IP3 to the sponge protein from knowledge of the binding of the derivative to IP3R. (As shown above, one does not know the binding affinity of a derivative at the 2-position based on the Hirata reference.)

Prior to the subject invention there was no convenient method for high throughput screening of IP3. The subject inventors were able to develop a robust assay with a stable tracer providing high sensitivity for IP3 specifically, without interference from other inositol phosphates. The methodology is simple, robust, easily performed in a high throughput context and provides for an accurate measurement of IP3 in a physiological sample. Compare the cumbersome procedure of Hirata that requires a filtration step and a radioactive tracer. It was not predictable, rather it was counter-indicated that the highly specific sponge protein would tolerate a derivative of IP3. Since the binding pocket was changed to provide the highly increased affinity of the sponge protein over the parent IP3R, it was to be expected that little if any modification of IP3 would be tolerated. Nevertheless, having attempted to use the sponge protein with the IP3 derivatives, a successful assay was developed. This could not have been foreseen. Faced with the challenge of developing such an assay with all of the relevant literature facing the inventors, it was not easy to conclude that despite the greatly enhanced binding affinity of the sponge protein, one could

derivatize IP3 and still obtain a robust assay, where the derivatized IP3 could successfully compete with IP3 for the binding site of the sponge protein.

In addition, none of the references are concerned with a real life situation. The candidates for binding to the IP3R are selected and controlled and the mixture of inositol phosphates encountered in native samples is not present. How the sponge protein would respond to such a situation is not shown in the literature.

Applicants should not be penalized for their success. Prior to their demonstration of the subject invention, it was not predictable that the components they chose could be put together to provide a sensitive assay that would not be subject to interference from other inositol derivatives. The assay must distinguish between IP3 and the other inositol derivatives, such as I-4,5 diphosphate, I-2,4,5 triphosphate, I-1, 2, 3, 4, 5, or 6 phosphate, I-1,3,4,5 phosphate, I-2,4,5 and the like. Modifying the IP3 at the 2-position changes the characteristics of the IP3 and would be expected to modify the binding of the IP3 derivative with the very specific sponge protein. If the derivatized IP3 had substantially lower binding affinity to the sponge protein than the IP3 there could be no assay and the other inositol derivatives would be capable of competing with the derivatized IP3 for the binding pocket of the sponge protein.

As for the "response to Argument," it is respectfully submitted that it does not meet what applicants previously argued. While Sportsman provides motivation, Sportsman does not provide an enabling disclosure in a situation that is fraught with impediments. The Response further states that it would be obvious to use the Iwasaki sponge protein with a 2-position derivatized IP3, because Hirata prepares such derivatives and shows that they can displace IP3 from a rat cerebellum homogenate. However, as amply demonstrated above, it is not clear what the various entities are binding to. Rather, there is little to take from the Hirata reference that would aid one in the decision to proceed in developing as assay using an IP3 derivatized at the 2-hydroxyl position and the sponge protein.

Finally, there is the rejection of claim 5, now canceled. It should be noted that the claims that were restricted were directed to enzyme fragmentation assays and claim 5 was more properly considered with those claims than the claims under consideration. It was deemed appropriate to cancel claim 5 and leave arguments for its patentability to the divisional application to be filed.

Conclusion

Applicants request that this amendment to the claims and specification be entered and requests reconsideration and allowance of claims 1-4, 6-8 and 18 for the reasons advanced above. In view of the above amendments and remarks the Examiner is respectfully requested to withdraw the rejections and pass this application to issue, canceling the withdrawn claims. If the Examiner believes that the prosecution of the application could be expedited by a telephonic interview, the Examiner is requested to call Bertram Rowland, Reg. no. 20,015, at 650 344 4674.

Respectfully submitted,

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